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Analysis of a structural motif in the membrane-associated [NiFe] hydrogenases

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Hydrogenases, catalyzing the following reaction: $2H^+ + 2e \Leftrightarrow H_2$, are harbored by numerous microorganisms. The cells dispose excess electrons through hydrogen production accomplished by hydrogenases, while consumption of the molecular hydrogen mostly provides electron source for various energy conserving processes, such as respiration. Sometimes, hydrogen can be the sole energy source for the cell growth. Hydrogenases are distinguished according to the metal content in their active center: they are classified as [NiFe], [FeFe] and [Fe] enzymes (Vignais et al. 2004). Minimally, a [NiFe] hydrogenase is composed of a large and a small subunit and they can be associated to the membrane or localized in the cytoplasm. The large subunit contains a binuclear metallocenter, while the small subunit hosting the Fe-S clusters, which conduct the electrons between the H_2-activating center and the surface of the protein.

Our model organism, the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina* BBS contains at least four active [NiFe] hydrogenases. The HynSL and the HupSL enzymes are attached to the cell membrane, while Hox1YH and Hox2YH are apparently localized in the cytoplasm (Kovacs et al. 2005).

There are several conserved motifs in the sequence of the hydrogenases, which are characteristic for these enzymes. These motifs have very important role for example in coordination of the metals of the active centre, in electron transfer, in interaction with other proteins or in translocation of the fully folded protein (Vignais et al. 2007).

We have noticed a highly conserved histidine-rich region with unknown function in the large subunit of [NiFe] hydrogenases. The HxHxxHxxHxH sequence occurs in the large subunit of all membrane-bound hydrogenases, but only two of these conserved histidines are present in the soluble hydrogenases.

In order to identify the function of this motif, mutant strains were made by site-directed point mutagenesis and their biochemical properties were characterized. The *in vivo* and *in vitro* activity measurements showed that the activity was influenced dramatically only in one of the mutants due to the replacement of the His residue with Ala. Nevertheless, this enzyme still remained in the membrane.

Western hybridization experiments were applied to investigate the proteolytic stability of the enzymes. It was found that the strongly reduced activity of the mutant hydrogenase could not be derived from the destabilization of the protein.

The oxygen sensitivity of the single amino acid mutant and the wild type protein was also compared for explaining the background of significant *in vitro* and *in vivo* activity loss in the mutant strain. The crude extracts from the wild type and mutant cells obtained in anaerobe box and on air was used for *in vitro* spectroscopic measurements, but the ratio of the activities of the wild type and the mutant protein was nearly the same independently on the environment of the cell destruction.

For further biochemical and biophysical investigations, large amount of enzymes have to be purified. First, a HynS-Strep-II Tagged fusion protein was constructed to purify the non-mutant HynSL enzyme by affinity chromatography. However the amount of the purified protein was not enough for further spectroscopic experiments and the specific activity of the Strep-II fused hydrogenase was lower as compared to the natural enzyme. We continued with the standard biochemical techniques and used fast protein liquid chromatography (FPLC) for purification of the wild type and mutant protein in large scale. These experiments are in progress and the first results are very auspicious.

For getting a complete picture about the structure-function relationship in HynSL hydrogenase, other conserved residues are being investigated, as well. From biotechnological point of view thermostability and oxygen tolerance of the hydrogenases are two crucial properties. In order to improve these properties of the enzymes, identification and functional characterization of candidate sequences potentially conferring these beneficial properties to the enzymes are to be done.

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Mechanistic insights into the role of translesion synthesis and its effect on genome stability

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REV1 is a Y-family DNA polymerase. REV1 proteins contain a BRCT domain, which is important in protein-protein interactions. A suggested role for REV1 protein is as a scaffold that recruits DNA polymerases involved in translesion synthesis (TLS) of damaged DNA.

To elucidate the mechanism by which REV1 promotes DNA damage bypass, we have analyzed the progression of replication on ultraviolet light-damaged DNA in mouse embryonic fibroblasts that contain a defined deletion in the N-terminal BRCT domain of REV1, or that are deficient for REV1.

DNA fiber labeling method that has been previously described has been adapted in which two modified nucleotides IdU and BrdU were used to label newly replicated DNA. Incorporated IdU and BrdU were detected by fluorescent immunolabeling and the progression of replication fork was monitored. To examine the effect of UV damage to DNA on replication fork progression, cells were treated with either 20J/m^2 or 40J/m^2 UV dose at the end of first labeling period (IdU) and before second labeling (BrdU). Fork rates were calculated for each labeling period and the ratio of IdU to BrdU were analyzed. Under normal replication conditions ratio of IdU to BrdU is approximately 1. However an increase in this ratio directly corresponds to the rate of fork stalling during second labeling as a result of UV damage to DNA.

To investigate the role of REV1 BRCT and REV1 in replication fork progression, DNA fiber spreads were prepared, labeled forks were measured and compared to that of wild type cell line. In wild type cells with no UV treatment the average ratio of IdU to BrdU was 1.13 and REV1 BRCT and REV1 mutant cells showed an average ratio of 1.13 and 1.16 respectively. There was no significant difference in the rate of fork progression in any of the mutant cell lines as compared to the wild type line. Therefore, these genes are dispensable for the normal growth and viability of the cell.

The frequency of fork stalling in REV1 BRCT and REV1 mutant cell lines after 20J/m^2 or 40J/m^2 UV dose was measured. Both REV1 BRCT and REV1 mutant cells showed a significant increase in the ratio of IdU to BrdU in response to 20J/m^2 UV treatment and the ratios increased further at 40J/m^2 UV. In wild type cells with 20J/m^2 and 40J/m^2 UV treatment the average ratio of IdU to BrdU increased from 1.13 to 1.92 and 3.24. REV1 BRCT and REV1 mutant cells showed an increase in the ratio from1.13 to 4.14 and 4.99, 1.16 to 3.43 and 4.5 respectively. Furthermore, the exogenous expression of mREV1 in REV1 BRCT and REV1 mutant cell lines restored wild type phenotypes.

These results provide an evidence for the role of BRCT domain of REV1 in response to DNA damage and that REV1 plays a central role in replication fork progression of UV-damaged DNA.

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Uptake and degradation of xenobiotic in *Sphingomonas subarctica* SA1

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Sulfanilic acid is a typical representative of sulfonated aromatic amines widely used and manufactured as an important intermediate in the production of azo dyes, plant protectives and pharmaceuticals. *Sphingomonas subarctica* SA1, a Gram-negative aerob bacterium is able to utilize sulfanilic acid as the only carbon, nitrogen, and sulfur source (Perei et al. 2001). In addition to sulfanilic acid, *Sphingomonas subarctica* SA1 could degrade six other aromatic compounds, such as sulfocatechol, protocatechol, para-amino benzoic acid, 3,5-dihydroxybenzoic acid and oil in soils.

Comparison of the protein patterns of cells grown on various substrates revealed that the strain used alternative metabolic pathways for biodegradation of these compounds. However, similar patterns were observed in the case of cells grown on sulfanilic acid and sulfocatechol. Therefore sulfocatechol is supposed to be formed, as first intermediate in the catabolism of sulfanilic acid. Unfortunately, sulfanilic acid could be converted by intact cells only but not by disrupted cells, thus the characterization of this reaction step was difficult. Nevertheless, sulfocatechol is further oxidized by a ring cleaving dioxygenase, named as sulfocatechol dioxygenase. This enzyme was partially purified and identified by mass spectrometry (Magony et al. 2007). A genomic locus harbouring the genes of sulfocatechol dioxygenase (*scaEF*) was also identified and upstream from these genes, few other *orfs* coding for proteins similar to muconate cycloisomerases (ScaA), lactone hydrolases (ScaB), maleylacetate reductases (ScaC) and an oxidase (ScaD) were recognized. These enzymes were actively overexpressed in *E. coli* and the sulfocatechol degradation pathway was reconstituted by the recombinant proteins.

The first step of sulfanilic acid degradation is not fully understood. The enzymes probably converting sulfanilic acid to sulfocatechol were very sensitive to cell disruption indicating that they were somehow related to the membrane. Proteomics approach was applied to identify of the enzymes catalyzing the sulfanilic acid conversion. Bands sepcifically appearing upon substrate induction in the membrane and soluble fractions were cut out and sequenced *de novo* by mass spectrometry.

The analysis of the proteomic data of the soluble fraction led to the identification of another gene set in the genome. In this locus, two genes likely coding for proteins involved in the oxidative deamination of sulfanilic were predicted.